

SULFURIC ACID HYDROLYZATES OF LOWLAND PEATS AS
GROWTH STIMULANTS OF MICROORGANISMS AND PLANTS.
III. AN INVESTIGATION OF THE COMPOSITION OF
ORGANIC ACIDS

G. A. Yevdokimova and G. I. Raytsina

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16. Abstract The grade of peat, H_2SO_4 concn. (in the range 2-30%), or reaction pressure (1 or 12-16 atm) did not affect the compn. of org. acids in the hydrolyzates (2-3.1 and 2.3-5.6%, resp. of volatile and nonvolatile acids). All of the hydrolyzates contained succinic acid (known for its high biol. activity), its concn. being a max. in the products obtained with 5% H_2SO_4 at high pressure. Paper chromatog. revealed the presence of nonvolatile oxalic tartaric, citric, glycolic, maleic, malonic, malic, glutaric, fumaric, adipic, and azelaic acids; of these, oxalic and succinic acids were significant.			
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Besides the amino acids, low molecular organic acids are a biologically active component of the hydrolyzates. Recently, works have appeared on a study of the composition of organic acids contained in the soils and water soluble substances of peats and their effect on plants [1 - 4]. There are no data on the quantitative and qualitative composition of the acids that are obtained by the action of mineral acid solutions on peat. Few studies have been devoted to a methodological treatment of the question of the composition of the acids of aqueous extracts and acid hydrolyzates of the plant raw material [3 - 5]. /91**

We investigated the composition of hydrolyzate organic acids obtained by the action of different concentrations of sulfuric acid on lowland peat, under atmospheric pressure and increased pressure [6].

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** Numbers in the margin indicate pagination in the original foreign text.

Method of investigation. The content of volatile organic acids was determined by distilling them from the hydrolyzates with the vapor and by subsequent titration with 0.1 n. NaOH. Measurement of the non-volatile acids was carried out in the following way: first, the amino acid fraction of the hydrolyzates was separated by the aid of the KU-2 cationite, as was described earlier [7], and the organic acids proved to be in the filtrate, which was evaporation-concentrated in a boiling water bath. The residue, consisting of a mixture of organic acids, was dissolved in 1 - 2 ml mixture butanol : chloroform (3 : 1), and the obtained solution was passed through a column with silica gel to separate the mixture of organic acids. Silica gel KSM, first pulverized and then strained through a sieve, was used in the work. A fraction of 0.1 - 0.2 mm was sampled. The silica gel was washed and activated by boiling for a period of five to six hours with distilled water, changed every 1.5 - 2 hours, and the gel was then dried at 100 - 102° C. The capacity of the silica gel was measured with respect to water absorption, and was 0.4 - 0.5 ml/g. The column (1 × 50 cm) was filled with moistened silica gel. During passage of the filtrate through the silica gel, the following acids were more stably retained: oxalic acid, tartaric acid, citric acid, and malic acid. These acids were very weakly extracted by the organic solvents, and were completely removed only by water. The silica gel also retained the dark, water soluble substances of the hydrolyzates, which made it possible to obtain pure fractions containing the organic acids.

The acids were first diluted with a mixture of solvents: n-butanol and chloroform in a ratio of 3 : 1 (fraction I), and then with water (fraction II). The thoroughness of removing the acids from silica gel was determined for each solvent by titration with 0.1 n. NaOH. The obtained eluates were vaporized in a vacuum; the residue was dissolved in alcohol. The acid numbers were measured in alcohol solutions, while the content of acids was calculated for succinic acid (fraction I) and oxalic acid (fraction II). The qualitative composition of the acids was established by the method of paper chromatography. Paper FN-3 was used for the work. Chromatography was carried out using a mixture of solvents; butanol-methanoic

TABLE 1. QUANTITATIVE COMPOSITION OF THE ORGANIC
ACIDS OF HYDROLYZATES*

T y p e o f p e a t	Concentration of sulfuric acid during hydrolysis, %	Acid yield, % of hydrolyzate organic substance		Characteristics of nonvolatile acids by fractions							
		Volatile	Nonvolatile	Fraction I				Fraction II			
				Fraction yield/hydrolyzate organic substance	Acid number, mg KOH/g of substance	% acid content/organic substance of fraction	% acid yield/organic substance of hydrolyzate	Fraction yield/hydrolyzate organic substance	Acid number, mg KOH/g of substance	% acid content/organic substance of fraction	Fraction yield/hydrolyzate organic substance
2	2.0	2.8	2.67	18.3	105.0	13.1	1.57	20.0	106.0	78	1.10
3	2.0	—	2.55	15.0	137.0	17.3	2.35	5.1	89.0	6.7	0.30
3	2.0	—	2.70	16.0	88.6	11.0	1.8	22.1	53.0	4.0	0.96
5	2.0	—	2.65	16.5	140.0	17.5	1.83	8.3	133.0	9.9	0.82
5	10.0	—	3.93	32.5	75.0	9.37	3.1	12.5	89.0	6.7	0.83
5	10.0	2.4	2.12	26.2	71.0	8.8	1.72	8.6	41.0	3.6	0.40
5	15.0	—	5.61	16.0	214.0	26.7	4.4	12.8	127.0	5.5	1.21
5	15.0	—	4.24	19.0	43.0	15.8	3.3	6.2	206.0	15.2	0.94
5	15.0	3.1	3.86	23.0	113.0	14.1	3.3	17.2	43.3	3.3	0.56
5	20.0	—	4.07	13.4	89.0	22.5	3.0	16.5	80.3	6.5	1.07
2	20.0	—	5.40	23.1	171.0	21.9	5.1	3.4	123.0	9.2	0.30
5	20.0	2.6	3.83	14.1	172.0	22.0	3.4	1.5	104.0	7.8	0.43
5	30.0	—	2.33	13.0	86.0	10.8	1.43	18.6	72.0	5.4	0.90
5	30.0	2.7	3.24	18.2	98.0	12.3	2.24	16.0	93.0	7.0	1.00

* Translator's note: Commas in numbers represent decimal points.

acid-water (2 : 1 : 2) in the descending stream. The chromatograms were developed using a 0.04% alcohol solution bromcreosol green, whose pH was quite alkaline, up to 7 - 7.5. The organic acids appeared in the form of yellow spots on a blue background. Identification of the acids was carried out according to R_f of the solutions of the pure organic acids.

Results of the investigation. Data on the quantitative and qualitative composition of the organic acids are cited in Tables 1 and 2; R_f of the acids are shown in Table 3. It follows from Tables 1

TABLE 2. QUALITATIVE COMPOSITION OF THE ORGANIC
ACIDS OF THE HYDROLYZATES*

Type of peat	Fraction I, taken from silica gel by a butanol-chloroform mixture (3 : 1)										Fraction II, taken from silica gel by water					
	Oxalic	Tartaric	Citric	Malic	Glycolic	Maleic	Maleonic	Succinic	Glutaric	Fumaric	Adipinic	Azelaic	Oxalic	Tartaric	Citric	Malic
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
35	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Under conditions of increased pressure

1	0.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	0.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	0.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1	0.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Note. (+) — slight amount; (++) — moderate amount; (+++) — high content.

*Translator's note: Commas in numbers represent decimal points.

and 2 that the type of peat and the concentration of H_2SO_4 (within limits of 2 - 30%) did not exert a marked effect on the quantitative and qualitative composition of the acids. The yield of acids per organic substance of the hydrolyzate, with a change in the concentration of H_2SO_4 , from 2 to 30%, comprised 2.0 - 3.1% for the volatile acids and 2.3 - 5.6% for the nonvolatile acids. All of the samples contained a significant amount of oxalic acid, which partially entered fraction I, but which was basically diluted with water. Traces of acid with a R_f value below 0.4 (Table 3) were found in fraction I.

Acids with a R_f value above 0.8 were either absent or were present in a small quantity.

A similar composition of acids was found in the hydrolyzates obtained at a pressure of 12 - 16 atm (Table 2).

TABLE 3. VALUES OF R_f OF THE ORGANIC ACIDS IN THE FOLLOWING SYSTEM OF SOLVENTS: BUTANOL - METHANOIC ACID - WATER (DESCENDING FLOW)

Organic acids	R_f	Organic acids	R_f
Oxalic	0.0 - 0.02	Succinic	0.66 - 0.68
Tartaric	0.16 - 0.17	Lactic	0.66 - 0.68
Citric	0.20 - 0.25	Glutaric	0.76
Malic	0.40	Fumaric	0.83 - 0.87
Glycolic	0.46 - 0.48	Adipinic	0.87
Maleic	0.57 - 0.58	Azelaic	1.00
Malonic	0.60		

All the hydrolyzates contained a small amount of succinic acid, which, as is known [8], has high biological activity. The highest content of this acid was noted in the hydrolyzates obtained by the action of 5% H_2SO_4 under increased pressure.

Thus, the hydrolyzates obtained by the action of different concentrations of sulfuric acid on lowland peat contain volatile (2.0 - 3.1% per organic substance of the hydrolyzate) and nonvolatile (2.5 - 5.8%) organic acids.

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Succinic acid, tartaric acid, citric acid, malic acid, glycolic acid, maleic acid, malonic acid, oxalic acid, glutaric acid, fumaric acid, adipinic acid, and azelaic acid were found in the composition of the nonvolatile acids by the method of chromatography. Oxalic acid and biologically active succinic acid are dominant among them.

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